

## **REMARKS**

### **Status of the claims**

Claims 1, 3-4 and 6-11 are pending in the application. Claim 1 is amended herein. Claims 2 and 5 were previously cancelled.

### **Rejections under 35 U.S.C. §103**

Claims 1, 3-4, 7-8 and 10-11 remain rejected under 35 U.S.C. §103 as being obvious over Chang et al. combined with Cook et al. and Sozzi et al.

Claim 6 remains rejected under 35 U.S.C. §103 as being obvious over Chang et al. in view of Cook et al. and Sozzi et al. and in further view of Wick et al.

Applicants traverse this rejection and withdrawal thereof is respectfully requested. In response to Applicants arguments of September 2, 2008, the Examiner asserts on page 2 of the current Office Action the following points.

- a) The Examiner points to column 2, lines 1-3 of Chang et al. for teaching the quantification of the expression of hTERT mRNA.
- b) The Examiner further points to column 10, lines 14-18 of Chang et al. for teaching the use of a reference curve in the determination of the quantification hTERT mRNA.
- c) Finally, the Examiner states that “The recitation as amended is not clear that the concentration of circulating total DNA in a plasma sample is determined by quantification of hTERT copy number. Therefore, the teachings of the references cited in the rejections satisfy the limitations of the claims.”

### **Distinctions between the instant invention and the prior art**

Claim 1, step 5, has been amended to more clearly recite that “quantifying the hTERT DNA copy number in the target DNA sample by interpolating a calibration curve created with known amounts of DNA, wherein the concentration of circulating total DNA in a plasma sample is determined by quantification of hTERT copy number.” Thus, claim 1, step 5, has been amended to explicitly recite that the concentration of circulating total DNA in a plasma sample is

determined by quantification of hTERT copy number. Claim 1 has been further amended solely for clarity purposes to place the claim in better form.

The Examiner acknowledges that Chang disclose the quantitation of expression of hTERT mRNA (page 2, par. 2), as opposed to the invention, which is based on the quantitation of hTERT DNA copy number. Additionally, the Examiner acknowledges that, unlike the present invention, the method of Chang is based on the detection of a cancer-specific marker, the aberrant expression of hTERT mRNA, i.e. hTERT mRNA that is not present in normal cells but only in cancerous cells (see e.g. claim 1: " a method for identifying the presence of cancerous cells in a human sample....").

However, the Examiner states that Chang in column 10, lines 14-18, discloses that "quantitation of a sample containing an unknown number of target sequences typically is carried out with reference to a standard curve generated from a series of amplification of samples containing the target sequence in a range of known amounts" and that "the recitation of Claim 1 as amended is not clear that the concentration of circulating total DNA in a plasma sample is determined by quantification of hTERT copy number", whereby the teaching of Chang satisfies the limitations of the claims. As noted above, claim 1 has been amended to clarify the differences in the disclosure of Chang and the instant invention.

In addition, there is a different conceptual approach and technical differences between the methods of Chang the instant invention. For example, the standard curve in the method of Chang is generated from "a series of amplification of samples", whereas in with the instant invention the standard curve is generated with a single sample (any type of genomic DNA) having a known amount of DNA.

The method disclosed by Chang comprises determining the quantity of hTERT mRNA in said sample and "in a control sample of non cancerous cells" and the comparison of the quantity of hTERT mRNA between the test sample and the control, non cancerous sample (see, e.g. claim 1). Thus with Chang the method is a comparative amplification and a typical example is provided in Fig. 4 and in Example 7 (column 24) of the reference. In contrast, the instant invention is an "absolute" amplification without the need of control (non-cancerous) samples for comparison because the instant invention does not examine a tumor-associated marker, but only

a variation in the amount of the total plasma DNA measured as the hTERT copy number in a plasma sample of unknown origin.

Moreover in the method of Chang, which consists of a quantitative amplification for gene expression (hTERT mRNA), in addition to the standard curve generated with serially diluted hTERT mRNA positive control samples, an additional reference standard curve using a suitable control gene (housekeeping gene) is required in order to estimate the initial mRNA concentration. Thus an "adjusted" hTERT mRNA concentration is calculated based on the expression of the housekeeping gene (column 21 line 25 to the bottom page; column 22, line 8-25; column 24 line 5-7 from the bottom page of Chang).

The secondary references fail to make up for the deficiencies in Chang. As such, the instant invention is not obvious over the cite references and withdrawal of the rejections is respectfully requested.

*Unexpected advantageous properties associated with the invention*

Attached hereto is a copy of "Sozzi G., Roz L., Conte a, Mariani L, Andriani F., Lo Vullo S., Verri C. and Pastorino U. Plasma DNA quantification in lung cancer computed tomography screening. Five years results of a prospective study", Published in Am J Respir Grit Care Med. 2009 Jan 1;179(1):69-74, which demonstrates the unexpected advantages associated with the instant invention.

Circulating DNA in plasma has been proposed as a useful marker for clinical management of symptomatic lung cancer patients but thus far no studies have evaluated the impact of biomarkers and DNA quantification in plasma with prospective spiral-Computed Tomography (CT) lung cancer screening trials. The instant inventors and Assignee of the presently claimed invention launched a prospective pilot trial of early lung cancer detection in Milan in 2000. The trial applied yearly low-dose spiral CT and selective use of positron emission tomography (PET) to a cohort of 1035 high-risk heavy smoker volunteers (minimum pack/year index of 20) aged 50 or older. The study also included systematic plasma sampling for DNA testing.

The inventors tested the methodology of plasma DNA quantification in a prior case-control study, which demonstrated higher levels of plasma DNA in 69% of 100 lung cancer patients versus only 2% of 100 matched controls (Sozzi G. et al JCO 2003), with a strong diagnostic power and a receiver operating characteristic (ROC) curve of 0.94. Given the high sensitivity and specificity of the qPCR test, that proved to be independent from tumor size, the inventors next decided to test the value plasma DNA quantification in combination with spiral CT for early lung cancer detection.

The Sozzi et al. (2009) article reports the results of plasma DNA quantification by hTERTqPCR in the entire cohort of 1035 volunteers enrolled in the early detection trial, based on a minimum follow-up of five years. Statistical analysis was carried out to investigate the diagnostic performance and the prognostic value of plasma DNA levels in a lung cancer screening setting.

Plasma samples were collected at first year of screening (baseline) and at the second year of annual screening (first spiral CT repeat) for all the 1035 volunteers. Additional blood samples were collected from the screened individuals when the CT results were indicative of lung cancer, as well as at the time of lung cancer resection throughout the study. Plasma separation, DNA extraction and quantification were performed as previously reported (Sozzi G. et al JCO 2003).

Screening performance of the assay was calculated through the area under the receiver-operating characteristic curve (AUC—ROC) and Kaplan-Meier analyses were computed for association with prognosis.

Of the 1035 subjects 956 remained cancer free over the 5 years of the study, 38 developed lung cancer, 2 developed a lung carcinoid and 41 developed other tumors. The clinical outcome of lung cancer patients was evaluated for a median follow-up period of 62 months. The median baseline DNA levels of lung cancer patients were not different from those of cancer-free controls, nor from the levels detected in patients with other tumors (AUCROC 0.496 ( $P=0.93$ ) and 0.492 ( $P=0.87$ ), respectively). A significant result was instead achieved by considering DNA levels at surgery in lung cancer patients, in which the AUC-ROC for the discrimination versus controls was equal to 0.607 ( $P=0.0369$ ). DNA levels at surgery were much higher for tumors detected in the first year of screening (median 10.1 ng/ml), with a trend towards a

reduction in year 2 (4.9 ng/ml) and years 3-5 (3.3 ng/ml,  $P=0.0141$  for the overall comparison) and tended to be higher for tumors in stages II-IV. In terms of diagnostic performance, these patterns translated into relatively high AUC-ROC figures when considering plasma DNA at surgery in tumors detected in the first year of screening (AUC—ROC 0.802,  $P<0.0001$ ), or Stage II-IV tumors detected during the first 2 years of screening (AUC—ROC 0.866,  $P=0.0001$ ).

Thirty-three patients had 2 to 4 plasma samples collected at different time points during the 5 years of the screening up to the time of surgery, for a total of 93 measurements. A significant difference ( $p=0.0010$ ) was observed in the amount of circulating DNA in plasma samples taken at all time points before surgery ( $n=60$ ; median 2.9 ng/ml) and those at the time of surgery ( $n=33$ ; median 4.7 ng/ml). The difference was still significant ( $P=0.0071$ ) when comparing DNA values in plasma samples collected within 12 months from surgery ( $n=57$ ; median 4.6 ng/ml) versus  $>12$  months preceding lung cancer diagnosis ( $n=36$ ; median 2.4 ng/ml), thus showing that plasma DNA tends to increase as the time to lung cancer diagnosis decreases.

In addition, an elevated concentration of circulating plasma DNA at surgery was associated with a shorter 5-years survival in resected patients. Tertile stratification showed that patients survival decreased for increasing plasma DNA at surgery ( $p=0.0066$ ). The worsening in survival (33% at 5 years) was evident in patients with DNA concentrations in the third tertile ( $\geq 6.3$  ng/ml). Because of the small overall number of deaths ( $n=11$ ), it was not possible to estimate the adjusted prognostic effect of plasma DNA with multivariable analysis. However, patterns similar to those described above were observed in the subgroups of patients with tumors detected in year 1-2, year 3-5, or in Stages IB-IV.

In conclusion the study presented in the Sozzi et al. (2009) article, showed an overall discriminatory power of plasma DNA levels for identification of lung cancer patients in a CT-screening setting. Interestingly, 5-year survival analysis, based on extended follow-up, demonstrates that a higher amount of plasma DNA at surgery might represent a risk factor for aggressive disease.

The diagnostic advantages associated with the invention are in no way suggested by the prior art references.


In view of the above amendment and Remarks, Applicants believes the pending application is in condition for allowance.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact MaryAnne Armstrong, PhD, Reg. No. 40,060, at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§1.16 or 1.17; particularly, extension of time fees.

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Respectfully submitted,

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**Attachment:** Sozzi et al. (2009) article